REMARKS

Claims 1-16 have been canceled. Applicants preserve all rights to file one or more divisional applications for the subject matter of claims 6-16. Claims 17-29 have been added.

The Examiner states that there is a discrepancy between SEQ ID Nos: 2 and 4 of the instant application and the priority application NO 2000 0163. SEQ ID Nos: 2 and 4 are the amino acid sequences that correspond to SEQ ID Nos: 1 and 2 of the original sequence listing. The PatentIn software automatically generates a separate sequence for the amino acid when the preceding sequence is for the DNA and the amino acid coded for by the DNA. A new sequence listing is attached.

UNG1 and UNG2 are nuclear and mitochondrial precursor variants of UNG. They are both encoded by the same gene but the two variants arise from differential splicing. This is a common feature for eukaryotic UNGs (Slupphaug et al. 1993). Both cDNAs are found in Cod liver, and their sequences are given. The two forms differ in the N-terminal sequences, which function as subcellular localization signals that are split off when entering their subcellular destinations. The resulting proteins are identical, and encompass the catalytic form of UNG. The native UNG that has been isolated in this work is presumably the nuclear form, but the two forms in mature state are, as described above, indistinguishable. (See page 17 of the specification).

The specification has been reviewed and no English spelling of hydrolysis was identified.

The Examiner has rejected claims 1 and 3-5 under 35 USC 112, first paragraph. Applicants respectfully traverse this rejection.

The Examiner states that absent a disclosure of the structural features common to the species within the genus, the description of two species is not sufficient as the description of the entire genus. Applicants respectfully disagree.

Support for the enzyme not being able to reactivate is found on page 7, lines 18-20 and lines 27-30 and page 30, lines 14-16 of the specification. Only two thermolabile UNG enzymes are known, and both of them are of microbial (prokaryotic) origin. The present invention

describes the first eukaryote UNG that also is thermolabile and not able to reactivate. It was highly unexpected to the inventors to find a thermolabile UNG that was not able to reactivate.

The main disadvantage of the available UNG enzymes is that even if they are completely heat inactivated, they reactivate. This is also the case for the commercially available thermolabile variant from the marine bacterium BMTU3346 (Sobek et al 1996). The inventors have performed a quantitative inactivation analysis based on the Sobek et al paper, which is presented below.

The process of reactivation requires that the protein structure is able to refold from a completely unfolded state. The refolding may result in a fully or a partially functional protein. This property is related to the protein primary structure, since the information necessary for refolding is contained in the amino acid sequence alone. It is also important to recognize that heat inactivation properties are functionally separate from refolding/reactivation properties. The first determines how quickly the protein unfolds, the second determines to what extent the protein is able to refold.

The primary sequence for UNG enzymes are particularly well conserved among vertebrates with 80-90% sequence identity in the catalytic domain between fish and mammals. The identity to UNGs from other phyla is at most 50%. The applicants have in this work found that the Cod UNG does not reactivate. Considering the high sequence identity within vertebrates, it must therefore be considered very likely that the primary structure (amino acid sequence) that is common to this group also determines the inability of enzymes from this group to refold.

The heat lability of the Cod enzyme is determined by a limited number of amino acid substitutions in the Cod enzyme compared to other vertebrate UNGs. This has been studied in detail in a thesis by Dr. Elin Moe, University of Tromsø, Norway. The results will be published shortly, but may be made available on request.

A measurement of residual activity/reactivation after thermal inactivation at 60°C was performed by the inventors.

Two PCR-tubes with both Units of reUNG (approximately 1.60 μg) in 40 μl "Taq-

buffer": (10 mM Tris-HCl (pH = 9.0 @ 25°C), 50 mM Kcl, 0.1% Triton X-100) was inactivated for 15 min at 60°C. After inactivation, both tubes were kept at 4°C. One tube was measured for residual activity one hour after inactivation, whereas the other tube was measured 19 hours after inactivation. Residual activity was measured by using 5 μl undiluted inactivated mix in a standard assay. Before inactivation, 2 μl of the enzyme mix was taken out and diluted 1:7000 before measurement of activity (100% control). The results showed that the inactivated enzyme did not cause release of radiolabeled uracil from the substrate that was above the detection limit (blank control + 2 x standard deviation). At these conditions, the 100% control would yield 26,000,000 cpm, while 2 x background standard deviation was 14 cpm. Consequently, the residual activity of rcUNG was less than 0.00005% of initial activity after thermal inactivation at these conditions. It was not any significant reactivation of the enzyme after 19 hours at 4°C.

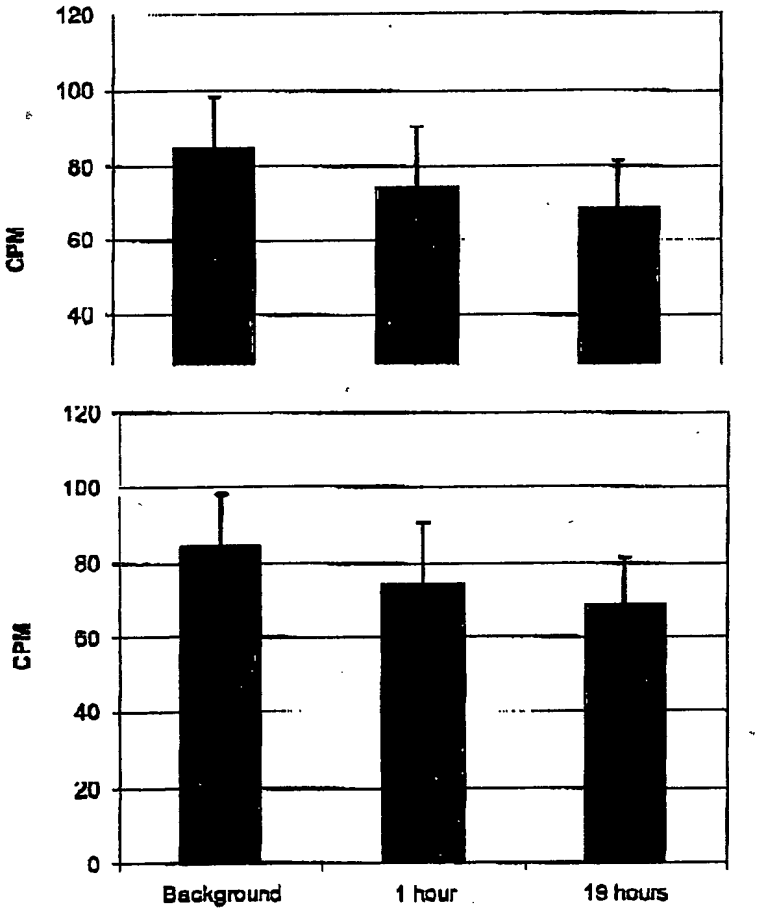

Figure 1: Residual cpm values of inactivated rcUNG samples 1- and 19 hours after inactivation compared to background cpm.

Quantitative inactivation analysis based on the Sobek et al, 1999 paper

UNG was added to a PCR reaction and samples were withdrawn at 0, 1, 2 and 16 hours. The extent of degradation of the PCR product where then analysed on a sequence gel, where the degradation product will appear as bands smaller in size than the original PCR product. Comparative experiments were performed with 2 units of UNG from BMTU (lanes A1-A4 in fig. 2 in Sobek et al, 1999) and E. coli (B1-B4). The controls were as follows: Negative controls; no UNG was added (lanes C1-C4), positive controls; BMTU UNG (D1-D4) and E. coli UNG (E1-E4) was added after the PCR reaction was completed.

On this basis the inventors were able to make semi-quantitative evaluations of the results. It is obvious when comparing A1-A4 with B1-B4 that UNG from BMTU yields less degradation of DNA than E. coli UNG. It is also obvious that BMTU UNG has a certain residual activity or reactivating as can be shown after 16 hours by 4°C (lane A4). The degree of degradation after 16 hours is nearly equivalent to what can be seen for E. coli UNC after 2 hours (lane B3). When comparing lane A4 with the positive controls (D and E), it is evident that the degree of degradation is insignificant lower in A4 than in D1 and E1 i.e. this corresponds to a degradation carried out by a 100% active enzyme during 1 hour.

From this experiment one can conclude that UNG from BMTU has an 8 times lower residual activity than UNG from E. coli after a PCR reaction (by comparing lanes A4 and D3). One can also conclude that the residual activity of heat inactivated BMTU UNG is approximately 5% of the full activity, since the degree of degradation of DNA after 16 hours with inactivated UNG is slight less than the degree of degradation after 1 hour with a fully active enzyme (comparing lane A4 with D1 and E1).

The inventors final conclusion is that UNG from BMTU is more inactivated than UNG from E. coli, but still there is a residual activity which is considerable regarding the areas of use for these enzymes.

Quantitative inactivating data from UNG from cod shows that the enzyme has less than 0.0006% residual activity after heat inactivation by 94°C in 10 min, in PCR buffer, the enzyme

is therefore completely inactivated under these conditions.

Therefore, based on the statements above concerning sequence identity and the failure of the enzymes to reactivate, it is clear that the inventors had possession of the invention at the time the application was filed. Therefore, it is respectfully requested that this rejection be withdrawn.

Therefore, it is respectfully requested that the rejection be withdrawn.

The Examiner reject claims 2-4 under 35 USC 112, second paragraph. Applicants respectfully traverse this rejection.

In view of new claims 17-29, it is respectfully requested that the rejection be withdrawn

The Examiner has rejected claims 1-5 under 35 USC 101. In view of new claim 17, this rejection is moot.

New drawings are being submitted with this response.

Accordingly applicants submit that this application is in condition for allowance and favorable consideration is respectfully requested.

Respectfully submitted,

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MARKED-UP COPY

Please replace last paragraph on page 7 with the following:

Preferably the enzyme according to the invention has an amino acid sequence as shown in SEQ ID NO[S]s: [1] 2 or [2] 4, or a biologically functional part thereof.

Please replace paragraph 2 on page 8 with the following:

A DNA-sequence encoding for the novel enzyme as defined in the patent claims is another aspect of the present invention. Preferably the DNA-sequence has a nucleotide sequence as given in the SEQ ID NO[S]s. [1] 2 or [2] 4.
The DNA sequence according to the present invention is preferably including a promoter and contained in an expression vector such as a plasmid, a cosmid or a virus.